PROGNOSTIC SIGNIFICANCE OF ARCHITECTURAL, INVASIVE AND PROLIFERATIVE FEATURES IN INVASIVE BREAST CARCINOMA

DISSERTATION

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CERTIFICATE

We hereby certify that the work embodied in the dissertation entitled **"Prognostic significance of architectural, invasive and proliferative features in invasive breast carcinoma"** is a record of work done by Dr. Sudha, in the Department of Pathology, Tirunelveli Medical College, Tirunelveli during her post graduate degree course in the period 2005 – 2008. This work has not previously formed the basis for the award of any degree or diploma.

Professor and Head of the Department

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CONTENTS

INTRODUCTION

Breast cancer has a major impact on the health of women. It is the most frequently diagnosed cancer and the second most common cause of cancer death in Indian women after cancer of the uterine cervix (Chopra R., 2001). Recently it is emerging as the commonest female malignancy in developing Asian countries ahead of carcinoma of uterine cervix. Presently 75,000 new cases occur in Indian women every year (Chopra R., 2001. loc.cit.).

The breast cancer has received a great deal of publicity and has been the focus of intensive study. Recently, there has been a slight decline in breast cancer mortality due to the early detection of breast cancer in stage I and stage II by mammography and effective cancer screening programme. This downward trend will continue, if better treatment modalities are followed.

Cytotoxic chemotherapy is a double-edged tool. It can increase life expectancy in advanced malignant disease as an adjunct to other therapy but also is a hazardous and potentially lethal form of treatment. Assessment of multiple prognostic factors is necessary to separate cases which benefit most from this form of therapy.

The clinical course of breast cancer varies considerably from patient to patient. The median survival of women with untreated breast cancer is 2 to 7 years

and 10-year survival, subsequent to the onset of symptoms is 3.6% (Francis E Sharkey, et al.,1996).

The pathologist has got critically important responsibilities as a consultant in the management of patient with breast cancer. The clinical evaluation estimates whether the cancer is localised to breast or whether there are regional or distant metastases. The pathologist, establishes the diagnosis of cancer and reports the significant characteristics which can be used in planning therapy by predicting the natural history of the disease and possible response to a particular mode of treatment. Another major reason for reporting pathological prognostic features is to monitor mammographic screening programmes.

So, this study is aimed at evaluating certain architectural, invasive and proliferative parameters in cases of infiltrating ductal carcinoma of breast.

AIMS AND OBJECTIVES

The aim of this study is to

1. Stage the cases of carcinoma breast based on the American joint committee on Cancer [AJCC] staging system
2. Assess the histological grade of the tumor on the basis of Modified Bloom – Richardson grading system
3. Identify the invasive, architectural and proliferative parameters in infiltrating ductal carcinoma – breast
4. Correlate clinical staging with the other, pathological prognostic factors.

REVIEW OF LITERATURE

Anatomy :

Breast is a modified sweat gland. It lies in the superficial fascia of the pectoral region. It extends from 2nd to 6th rib and from sternal edge to near the mid axillary line. A small extension called the axillary tail of Spence pierces the deep fascia and lies in the axilla. Approximately three quarters of the breast is on the pectoralis major muscle normally.

Histology :

The functional unit of the breast is called a lobule. There are numerous lobules within each breast. A lobule consist of a variable number of acini (glands) lined by a double-layered epithelium.

- Outer flattened myoepithelial cell

- Inner cuboidal cell.

The acini drain into a terminal duct. Each terminal duct and its acini are together referred to as the Terminal Duct Lobular
Unit (TDLU). The terminal duct drains into subsegmental duct, segmental duct, and finally into lactiferous duct. There are 15 – 20 Lactiferous duct which open into the nipple. Immediately below the nipple, the lactiferous duct dilate to form

the Lactiferous sinus. The collecting ducts are lined by 2 distinct type of cells – basal myoepithelial cell and luminal columnar cell.

Breast carcinoma can occur at any age, but is rare in patients younger than 25 years. The peak incidence is 45 – 60 years. It is conventional to subdivide carcinoma of the breast into two main pathologic categories – in situ carcinoma and invasive carcinoma.

American Joint Committee on cancer, divided breast carcinoma into clinical stages as follows.

Stage 0 : Carcinoma in situ

Stage I : Invasive carcinoma $\leq$ 2 cm in diameters without nodal involvement.

Stage II : Invasive carcinoma $\leq$ 5 cm in diameter with upto three involved axillary nodes (or)

Invasive carcinoma greater than 5 cm without nodal involvement.

Stage III : Invasive carcinoma ≤ 5 cm in diameter with four or more involved axillary nodes (or)

Invasive carcinoma greater than 5 cm in diameter with nodal involvement;

Invasive carcinoma with 10 or more involved axillary nodes;

Invasive carcinoma with involvement of ipsilateral internal mammary lymph nodes;

Invasive carcinoma with skin involvement, Chest wall fixation or clinical inflammatory carcinoma

Stage IV : Breast cancer with distant metastasis.

For a number of tumors, the role of histopathology in the provision of prognostic information has been well established for many years. The main reason for this attempted sub classification has been the availability of a range of therapies and the need to stratify patients appropriately. In the last decade, the treatment of breast cancer has undergone dramatic changes and a much wider range of therapeutic options are now available. Early diagnosis, since the advent of breast screening, is detecting tumors which are likely to have a favourable out come and it has become increasingly important to assess prognosis for each patient

before a therapeutic plan is agreed.

The following pathologic factors, have been shown to provide clinically useful prognostic information to a greater or lesser degree.

1) TUMOR SIZE:

The diameter of the primary tumor shows a good correlation with the incidence of nodal metastases and with survival rate. This easily, quickly and cheaply determined parameter has been found to be one of the strongest predictor of dissemination and rate of relapse in node negative breast carcinoma. Women with node negative carcinomas under 1 cm in diameter have a prognosis approaching that of women without breast cancer (Rosai and Ackerman's Surgical Pathology., 2004). The 10-year survival of such women without treatment is approximately 90%. On the other hand, over half of women with cancers over 2 cm in diameter present with lymph node metastases and many of these women will eventually succumb to breast cancers (Susan C Lester,et al., 2004).

For correlation with prognosis, the size of tumors should only be assessed on pathological specimens, as clinical measurement may be inaccurate. Measurement of tumor diameter in three planes should be made to the nearest millimeters, initially in the fresh state. After fixation , when the edges of tumor have become

more clearly defined, the measurements are rechecked and the greatest diameter is taken as the tumor size. For small tumors and in cases where there is doubt about the definition of tumor margin, the measurement should be further assessed on the histological sections using the vernier scale on the microscope stage micrometer.

Patients with small tumor have a better survival than those with large tumors (Ellis IO, et al. 1996). Significant correlation was found by Elston CW, et al., (1982), Fisher ER, et al., (1984) Neville AM, et al., (1992).

In the long term study from the Memorial Sloan–Kettering Cancer Centre the projected relapse-free survival rates for 20 years after initial treatment were as follows (Rosen PP, et al., 1990) :

Tumors size	**20 yrs relapse free survival rate**
< 10 mm	88%
11 – 13 mm	73%
14 – 16 mm	65%
17 – 22 mm	59%

Analysis of size categories in the Nottingham Tenovus study indicates that for long term survival the better cut off point is actually at 1.5 cm (Elston CW, et al., 1998). In the study by Palmer MK, et al., (1982), patient with a breast tumor 2

cm or less in maximum diameter had a significantly better survival than patients with larger tumor. Rosen PP, et al., (1981) suggested that tumor larger than 1 cm is associated with a significantly poorer prognosis. Study by Gohari MR, et al., (2006) shows that patients with tumor size larger than 5 cm were in higher risk of metastasis compared with others.

2) Excision Margins :

Microscopic examination of the excision margins is usually undertaken to assess the adequacy of surgical excision and hence the probability of recurrence. The excision margins are usually marked with India Ink, which may track along the interstices of the specimen through defects in the surface. It has been found in most studies that when tumor reaches the excision margins, there is a significantly increased risk of local and distant recurrence (John P. Sloane., 2001).

Schnitt SJ, et al., (1994) in their study defined three categories of adequacy of excision (i) positive, in which tumor was present at the inked margins; (ii) close - in which the tumor was within 1mm; and (iii) negative - in which the tumor was more than 1 mm clear. The 5 year recurrence rate for those with negative, close, focally positive and more than focally positive margins was 0%, 4%, 6% and 21% respectively. Patients with positive margins have involved lymph nodes at presentation and have distant metastasis at 5 years, suggesting that margin involvement may be a manifestation of more aggressive disease.

Fourquet A, et al., (1989), found the gross margin involvement to be a major risk factor for local recurrence after local excision in multivariate analysis even when adjuvant radiotherapy is given.

Wazer DE, et al., (1997) in their study, concluded that the relative closeness of tumor to the specimen edge and the extent of margin positivity are predictive of residual tumor, though with an error consistent with its limitations as a sampling procedure. The histopathology of tumor in the initial excision is predictive of the type of residual tumor and the extent of margin positivity was correlated with the amount of residual tumor.

The extent of positivity of the excision margin was graded by Wazer DE, et al., (1999), according to a four – point scale : focal, minimal, moderate, extensive. They suggested that an extent of positivity grade of moderate / extensive is a predictor for local recurrence after breast conservation therapy.

Curcio LD, et al., (1999) study suggested that negative surgical margin in inflammatory breast cancer and nonmetastatic disease, is associated with a better overall outcome.

3) Histological Types :

Mammary epithelium gives rise to an assortment of carcinoma that number between 20 and 30. Classification of these tumors have significant role in prognostication. (Gallagen HS., 1994) In 1968, first edition of "Histological typing of breast tumors" by WHO was formulated (Scarff RW, et al).

The most common histologic types of Invasive breast carcinoma are (Dixon JM, et al., 1985).

Histologic types		Distribution
No special type Carcinoma ("ductal")	-	79%
Lobular Carcinoma	-	10%
Tubular / Cribriform carcinoma	-	6%
Mucinous (Colloid) Carcinoma	-	2%
Medullary Carcinoma	-	2%
Papillary Carcinoma	-	1%
Metaplastic carcinoma	-	< 1%

Other rare types of carcinoma,

- Apocrine carcinoma,
- Carcinoma with neuroendocrine differentiation
- Clear cell carcinomas
- Others

Specific features characterise some breast cancer, while the remaining which

lack these special features are termed invasive ductal carcinoma of No Special Type (NST). It includes the majority of carcinomas (70% to 80%) that cannot be classified as any other subtype (Susan C Lester, et al., 2004. loc.cit). The survival data for ductal carcinoma (NST), at 10 years ranges from 33 to 48% (Ellis IO, et al., 1992).

Infiltrating lobular carcinoma comprises about 10% of invasive breast carcinoma (Thomos J Anderson, et al., 1992). The cells are small and regular. There is characteristically single cell infiltration, often in a single file. It has a better prognosis than ductal NST carcinoma (Wheeler J.E., et al., 1976). In a study of 1621 patients with invasive breast carcinoma, Ellis IO, et al (1992 loc.cit) found that the 10-year survival for those with lobular Carcinoma was 54%.

Tubular carcinoma is a low grade breast carcinoma and has well formed infiltrating glands with a single cell lining and little cytological atypia with abundant stroma. Patients with pure tubular carcinoma have an excellent prognosis. Cooper HS, et al., (1985) recorded a 15 year survival of 100%. In the study of Ellis IO, et al., (1992 loc.cit) the 10-year survival was 90%.

Cribriform carcinoma has evenly distributed intraepithelial spaces which are regular in shape. Venable JG, et al., (1990) found that the 5-year survival with infiltrating cribriform carcinoma was 100%. The long term survival with invasive

cribriform carcinoma appears to be atleast 90% (Ellis IO, et al., 1992. loc.cit).

Mucinous carcinoma has cells floating in mucin pool. Pure mucinous carcinoma carry a very good prognosis and the 10-year survival data varies between 68% and 90% (Ellis IO, et al., 1992. loc.cit; Clayton F, et al., 1996).

Medullary carcinoma is characterised by a solid syncytium like sheets of large pleomorphic tumor cells with a rich lymphoplasmacytic infiltrate and a pushing border. Medullary carcinoma of the breast carries a good prognosis. Ridolfi RL, et al., (1977) found an overall 84% 10-year survival for medullary carcinoma. Similar results have also been recovered by Wargotz ES and Silverberg SG (1988). In contrast with these results, a number of other studies have failed to demonstrate a significant survival advantage for medullary carcinoma (Fisher ER, et al., 1990). Ellis IO, et al., (1992) loc. cit therefore concluded medullary carcinoma should be regarded as having a moderate prognosis.

Invasive papillary carcinoma with papillary architecture is a very rare tumor. Fisher ER, et al., (1980) and Mc Divitt RW, et al., (1968) concluded that it carries a favourable prognosis.

Metaplastic carcinoma – a group of tumors in which the malignant epithelial cells show evidence of metaplasia to mesenchymal cells or epithelial cells not normally found in the breast, is a rare tumor. It behaves as highly malignant

tumors with early recurrence and poor survival (Ellis IO, et al., 1996. loc.cit).

In summary, patient with the common histological types of breast carcinoma may be stratified into four broad prognostic groups (Ellis I, 1997).

Rating Prognosis	10 – yr survival	Type of Carcinoma
Excellent	> 80%	Tubular, Cribriform, Mucinous
Good	60 to 80%	Mixed ductal NST with special type
Moderate	50 – 60%	Medullary, Invasive Papillary, Lobular
Poor	< 50%	Ductal NST

4) Vascular Invasion

Tumors stimulate the growth of host blood vessels, a process called angiogenesis, which is essential for supplying nutrients to the tumor. Vascular invasion is more common in this situation and large veins may be involved by intravascular growth of tumor. Penetration of small lymphatic and blood vessels is associated with a poor prognosis and involvement of large veins with intravascular extension of tumor have the potential of releasing tumor cell aggregates or emboli into the venous circulation. These large tumor cell aggregates have been demonstrated to be associated with a higher efficiency of metastasis formation and infer a poorer prognosis. Many malignant cells are being released into the

circulation of cancer patients and few, if any ever successfully complete the complex sequence leading to a metastatic focus. This has been termed “metastatic inefficiency”. The finding of occasional small aggregates of single tumor cells in vascular spaces statistically implies that a considerable number of cells must be entering the vascular compartment (Crissman JD., 1986).

Conflicting view have been expressed concerning the prognostic value of estimating vascular invasion in breast cancer. Some studies have found no correlation (Dawson P, et al., 1982) while others have shown that the presence of vascular invasion predicts both recurrence and long-term survival. It may be due to the wide variation in the reported frequency of vascular invasion and the related problem of the distinction of true vessels from artifactual soft tissue spaces (Ellis IO, et al., 1996. loc.cit.).

Tumor emboli are mainly seen within thin walled channels. Since it is almost impossible to determine whether such spaces are lymphatics or venules the broad term “vascular invasion” is used (Elston CW, et al., 1998. loc.cit). The major problems lie in over looking small tumor emboli in capillary vessels and distinguishing them from masses of tumor exhibiting retraction from the surrounding stroma as a result of processing artifact (John P. Sloane. 2001). These problems can be greatly reduced by obtaining good fixation and by working to

simple but strict criteria. The determination of vascular invasion should only be made in tissue adjacent to the tumor mass and not within it. Tumor emboli must be seen within spaces having a clear lining of endothelial cells; these spaces are usually located within connective tissue separated from mammary lobular elements by interlobular stroma and are often in close proximity to small muscular blood vessels (Elston CW, et al., 1998. loc.cit).

Examination of hematoxylin and eosin stained sections is the most reliable method for identifying vascular invasion in breast cancer (Pinder S, et al., 1994). Reticulin stain can be used. Immunostaining for endothelial markers [CD 31 and CD 34] should be reserved for equivocal cases (Bettelheim R, et al., 1984b).

Most researchers have found a significant relationship between the presence of vascular invasion and prognosis as judged by local recurrence, disease-free survival or overall survival (Pinder S, et al., 1994. loc.cit; Davis B.W. 1985) . Vascular invasion has also been shown to be a predictor of lymphnode status, tumor grade and size, but not of estrogen receptor status (Pinder S, et al., 1994. loc.cit; Orbo A, et al., 1990). Vascular invasion also shows a high correlation with tumor type, development of distant metastasis and poor prognosis (Lee AKC, et al., 1986). Weigand RA, et al., (1982), examined blood vessel invasion and axillary lymph node involvement in 175 breast cancer patients and concluded that blood vessel invasion is a useful indicator of early recurrence in patients with

primary breast cancer and in combinations with node status is a prognostic indicator with high discriminatory power.

Van den Eynden GG, et al., (2006) used anti-CD34 as pan endothelium marker and demonstrated that it is possible to distinguish between blood vessel invasion and lymph vessel invasion in breast cancer specimens. In multivariate analysis, peritumoral lymph vessel invasion was the only independent determinant of lymph node metastasis.

5) Lymph Node Stage :

Axillary lymph node status is the most important prognostic factor for invasive carcinoma in the absence of distant metastasis. The clinical assessment of nodal involvement is very inaccurate with both false positive findings [eg. palpable reactive nodes] and false negative findings [eg., lymph nodes with small metastatic deposits]. Therefore, biopsy is required for accurate assessment (Susan C Lester, et al., 2004. loc.cit).

Numerous studies have shown that patients who have histologically confirmed loco-regional lymph node involvement have a poorer prognosis than those without nodal involvement. On average, 10-year survival is reduced from 75% for patients with no lymph node involvement to 25 – 30% for those with lymph node metastasis (Carter GL, et al., 1989; Veronesi U, et al., 1993).

Prognosis is also related to the overall number of loco-regional lymph nodes involved (Elston CW, et al., 1998). Fisher ER, et al., (1978), in their study have also concluded that survival is more likely related to the number of nodes involved rather than the size of the deposit. For prognostic purpose the best grouping seems to be the following (Rosai and Ackerman's Surgical Pathology., 2004 loc. cit.).

1 – Negative nodes.

2 – One to three positive nodes

3 – Four or more positive nodes

With no nodal involvement the 10-year disease-free survival rate is close to 70% to 80%; the rate falls to 35% to 40% with one to three positive nodes and 10% to 15% in the presence of more than 10 positive nodes (Susan C Lester, et al., 2004. loc.cit).

The level of nodal involvement also provides useful prognostic information; metastasis to the higher level in the axilla and specifically the apex, carriers a worse prognosis. Careful manual dissection of fixed axillary adipose tissue is the most cost effective method for isolating lymph nodes for microscopic examination (Rosen PP. 1991).

In the study by Jatoi I, et al., (1999) they concluded that nodal metastasis is not only a marker of diagnosis at a latter point in the history of breast cancer, but also a marker of aggressive phenotype.

6. Histological grade :

Greenhough RB(1925.loc.cit) was the first to evaluate histological grading in breast cancer by assessing eight different morphological factors – the amount of gland formation, the presence of secretory vacuoles, cell size, nuclear size, variation in the size of both cells and nuclei, the degree of hyperchromatism and the number of mitosis. Scarff RW, et al., (1928.loc.cit) emphasised on the amount of tubule formation, inequality in the size of nuclei, hyperchromatism. The mitotic figures was considered to be of less importance.

In 1957, Bloom HJG, a radiotherapist and Richardson, a surgical research fellow introduced a numerical scoring system in histological grading; Each of the three factors – tubule formation, nuclear morphology and mitosis, was scored on a scale of 1 to 3, giving a possible total score of 3 to 9 points. Grade was allocated by an arbitrary division of the total points.

This Patey and Scarff method, modified by Bloom and Richardson was adopted as the preferred grading system by the WHO (Ellis IO, et al., 1992 loc.cit). The statistical analysis shows that the WHO – grading proves to be the most indicative prognostic factor next to the number of affected lymph nodes and tumor size (Schnurch HG, et al., 1985).

Black MM, et al., (1955) re-evaluated the method which Bloom HJG (1950)

advocated and concluded that nuclear morphology was most important. Since both architecture (Bloom – Richardson system) and cytology [Black] have been found to correlate with prognosis, the sensible proposal has been made to use them in conjunction (Lash RH, et al., 1986). Elston has been the champion of this approach, which is usually referred to as the Nottingham modification of the Bloom – Richardson system and which also incorporates the evaluation of mitotic activity (Elston CW, et al., 1990; Frierson HF, et al., 1995).

The grading criteria for this system are:

i). Tubule formation :-

Only structures with a clearly defined lumen, indicative of ductal or glandular differentiation are included. Spaces formed as a consequence of other mechanism, such as poor cell cohesion or cellular necrosis are excluded. The following percentages refer to the area of the carcinoma exhibiting tubule formation for each of the three scores :

1 = more than 75%

2 = between 10% and 75%

3 = Less than 10%

ii) Nuclear pleomorphism :

In order to introduce a degree of objectivity, the size and shape of normal epithelial cells present in breast tissues adjacent to the tumor should be used as a

reference point. If normal epithelial structures are not present in the tumor section, then it is usually possible to find inflammatory cells such as lymphocytes for comparative purpose. Allowance should be made for the fact that lymphoid cells have a relatively smaller overall size than epithelial cells. The following criteria are used :

- 1 = The nuclei are small and exhibit little increase in size over that of normal breast epithelial cells. They have regular outlines and uniform nuclear chromatin and show little variation in size.
- 2 = The cells are significantly larger than normal with open vesicular nuclei, discernible nucleoli and moderate variability in size and shape.
- 3 = The nuclei are large and vesicular, often with prominent nucleoli, show a marked variation in size and shape and occasionally exhibit very large and bizarre forms.

iii) Mitotic counts :-

Hyperchromatic nuclei is more likely to indicate individual cell necrosis (apoptosis) than proliferation, and such nuclei should be excluded from the counts. Only figures, which clearly fulfill the morphological criteria for the various stages of mitosis – prophase, metaphase, anaphase and telophase, are included in the count (Elston CW, et al., 1998. loc.cit).

A minimum of 10 fields is counted at the periphery of the tumor where it has

been demonstrated that proliferative activity is greatest (Verhoeven D, et al., 1990).

A score of 1 is given for a count of 5 or fewer mitosis per 10 high power fields. Score of 2 for a count of 6-10. A score of 3 for more than 10 (Elston CW, et al., 1998 loc.cit.).

Allocation of grade :

To obtain the overall tumor grade the scores for each factor are added together giving a possible total of 3 – 9 points. Tumor grade is then allocated on the following basis.

Points	**Grade**
3 – 5	I – well differentiated
6 – 7	II – moderately differentiated
8 – 9	III – poorly differentiated

7. HORMONE RECEPTORS :

Carcinoma of the breast is often responsive to hormones, a property which has been exploited through endocrine surgery and more recently using drugs which influence hormone levels or inhibit the effects of hormones on tumor cells (Elston CW, et al., 1998 loc.cit).

Steroid receptors such as estrogen and progesterone receptor are located in the cell nucleus. Hormone is believed to diffuse or be transported to the nucleus where a steroid receptor complex is formed. Some of the genes regulated by steroid receptors are involved in controlling cell growth and it is currently believed that these effects are the most relevant to estrogen receptor influences on the behaviour and treatment of breast cancer (Elston CW, et al., 1998. loc.cit.).

Tamoxifen, an anti-estrogenic drug is the most widely used agent and it acts by inhibiting the action of hormones in their target tissues. The demonstration that radio-labeled estradiol is bound to some breast cancer specimens and that this effect was related to response to hormone ablation led to the development of hormone receptor assays directed at identification of patients suitable for hormone therapy.

Estrogen Receptor (ER) and Progesterone Receptor (PR) assay helps in predicting the response to hormone therapy (Cancer, 1980).

ER positive PR positive tumors have a 78% response

ER negative PR positive tumors have a 45% response

ER negative PR negative tumors have a 10% response

Evidence of significant benefit from endocrine treatment has been observed in patients whose tumors contain as few as 1% positive cells (Clark GM, et al., 1997). Elledge RM and Osborne CL (1997) have argued that when the cut-off

point is stringently low and the assay is of high quality, patients with ER-negative tumors will experience little or no benefit from tamoxifen, especially when it is used as adjuvant treatment, significant financial saving can thus be made by not using the drug indiscriminately.

The cytosol ligand binding assay has until recently been the standard assay method but has a number of disadvantages - large amount of tissue is required and is affected by high endogenous levels of estradiol. With the development of monoclonal antibodies specific for the receptor protein, the immunocytochemical method has superseded the ligand binding assay, as they require less tissue, allow formal histological assessment (King WJ, et al., 1984; Goulding H, et al., 1995) and may be used on very small sample such as fine needle aspirates (Robertson JFR, et al., 1992).

Rich MA, et al., (1978), Hahnel R, et al., (1979) and Aamdal S, et al., (1994) in their study suggested that the estrogen receptor content of human breast cancer specimen is related to the degree of differentiation (grade) of the tumor. In addition, patients with estrogen receptor – positive tumors experience fewer recurrences and remain disease free for a longer period of time than do patients with receptor negative tumors. But this advantage gradually disappeared with increasing interval after mastectomy.

Chang JC, et al., (1981) study suggest that ER+ status has a beneficial effect

in the responsiveness of advanced breast cancer to chemotherapy of Cyclophosphamide, Methotrexate and 5-Fluorouracil (CMF) and is prognostic of better survival. Clark GM, et al., (1983) found that PR was more important than ER in predicting disease-free survival for a group of patients with stage – II breast disease. Bernoube A, et al., (1998) in their study concluded that the disease-free interval and the metastasis-free survival tended to be worse for ER –/ PR – than for ER – / PR+ patients during the first 5 years.

8. ARGYROPHILIC NUCLEOLAR ORGANIZER REGIONS (AgNORs) :

The degree of malignancy of an individual neoplasm cannot be predicted with certainity by simple microscopic analysis alone. The criteria by which degree of malignancy is assessed [eg. nuclear pleomorphism, nucleocytoplasmic ratio, polarity loss and mitotic frequency] are often unsatisfactory in individual tumors. Accordingly newer methods have been developed for describing malignancy more objectively which include :-

i). DNA flow cytometry – the amount of DNA per nucleus is assessed.

ii) Labeling replicating cells with monoclonal antibodies like Ki – 67; BK 19.9.

iii) The AgNOR technique.

Nucleolar organizer Regions (NORs) are specific portions of DNA, called rDNA that, by using the enzyme RNA-polymerase-1, code for the transcription of

ribosomal RNA (rRNA) (Crocker J, 1992). This latter then forms ribosomes and these ultimately assemble proteins . The NORs can be seen close to the centromere as a ‘secondary constriction’ in conventionally prepared chromosomes or by hybridization methods, but are usually demonstrated by the binding of their associated protein to silver [Ag^{++}] ions. The chromosome preparations, cells or paraffin tissue sections are exposed to a silver formate colloid stabilized by gelatin, which enables the Ag^{++} ions to bind to the Nucleolar organizer Regions Associated Proteins [NORAPs] – a technique described by Good pasture and Bloom in 1975, modified by Ploton, et al., in 1986. This is called ‘AgNOR’ reaction and all reaction sites ‘AgNORs’ (Crocker J, 1992.loc.cit.).

This technique is very simple and does not require special preservation or fixation of tissue. It can be performed on formalin – fixed, paraffin – embedded section (Ajay K Khanna, et al., 2005).

Silver stained slides were examined under light microscope at 100x magnification AgNORs appear as brown or black dots within a yellowish background of nucleus. The number of dots in 100 cells should be counted and the average is often referred to as mAgNOR (mean AgNOR) (Ajay K Khanna, et al., 2005). The number of AgNOR rises with the increasing proliferative activity of cells (Morgan DW, et al., 1998). Thus, compared to normal or benign lesions, the number of AgNOR dots in malignant lesions is higher (Uno T, et al., 1988). Due to

the simple quick and convenient nature, this method has been used in histopathological diagnosis of various benign and malignant human tumors ,such as breast prostate and salivary gland tumors (Ghomette GP, et al., 1991; Morgan DW, et al., 1998 loc.cit).

Most AgNOR studies focus on the difference in AgNOR counts among tumors of different pathological grades and tissues in different stages of neoplasia i.e. dysplasia ,in situ carcinoma or invasive carcinoma (Mulazim Hussain Bukhari, et al., 2007). Roller E, et al., (1993), in their study found a clear cut difference between benign breast diseases as compared with breast carcinomas where the mean AgNOR count was significantly higher.

9. Nottingham Prognostic Index :

Until comparatively recently, the only factor used consistently as a guide for therapy has been locoregional lymph node status. Lymph node status is a time-dependent prognostic factor and the longer a tumor has been growing the more likely it is that spread to lymph nodes have occurred. Although lymph node status is a ‘powerful factor’, it takes no account of the innate aggressiveness of tumors.

The aggressiveness of a tumor depends on a number of biological characteristics like differentiation, growth rate, hormone responsiveness. If accurate prognostication is required on an individual patient basis then a

prognostic index is required which uses both time-dependent factors and biological factors.

Greenhough RB (1925 loc.cit.), he noted that the combination of high grade malignancy and lymph node involvement gave an exceedingly poor prognosis. Bloom HJG (1950 loc.cit.) too stressed that prediction of survival was improved by combining grade with stage. The 5-year survival of 94% for patients with grade I tumors and uninvolved nodes fell to 65% for those with involved nodes and for patents with grade III tumors from 55% to 16%. Similar findings were subsequently reported by Elston CW (1982 loc.cit.).

In a study of nine putative prognostic factors in nearly 400 patients with primary operable breast cancer, Haybittle JL, et al., (1982) found that several showed a significant relationship to prognosis, but only three – size, grade and lymph node status – remained significant on multivariate analysis. Using the coefficients of significance for these three factors, an index predicting survival, that become known as the Nottingham Prognostic Index [NPI] was calculated :

$$NPI = 0.2 \times \text{size (in cm)} + \text{lymph node stage [1-3]} + \text{grade [1-3]}$$

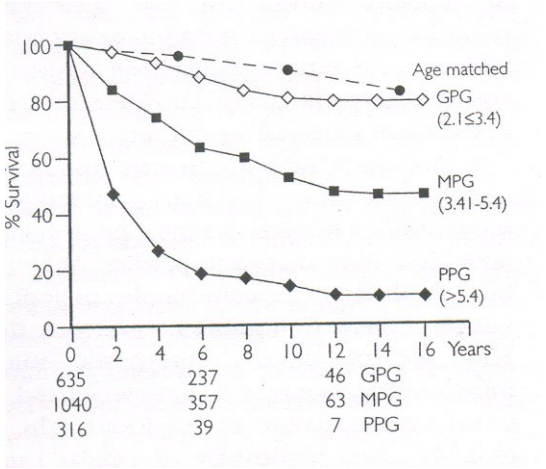
The higher the value for NPI the worser the prognosis. Curves of survival by life table analysis showed excellent separation of patient groups, depending on the index value. Three groups of patients have been identified by employing (arbitrary) cut – off points.

Prognosis	NPI
Good	≤ 3.4
Moderate	*3.41 – 5.4*
Poor	≥ 5.41

A number of subsequent prospective studies have been undertaken to review the efficiency of the index in predicting the clinical outcome of an increasingly large number of patients with increasingly long follow-up times [Todd, et al., 1987; Grade, et al., 1992]. The graph:1 shows a recent update of the Nottingham services. An excellent prognostic group has also been recently defined to take account of patients whose tumors have been detected by screening.

Galea MH, et al., (1992) gave a simplified therapeutic guide based on the index. The efficiency of the index has been confirmed by workers in other centers (Baslev I, et al., 1994). The main strength of NPI lies in the fact that it is based on relatively simple data which can be provided in routine histopathology laboratory.

Graph1: showing survival according to Nottingham prognostic index.



GPG= good prognostic group

MPG= moderate prognostic group
PPG= poor prognostic group

MATERIALS AND METHODS

This study was carried out in the Department of Pathology, Tirunelveli Medical College, Tirunelveli, during the period of December, 2005 to April, 2007.

A total number of 80 cases of breast carcinoma who have undergone mastectomy with axillary clearance was included in the study .

The age group varied from 24 years to 76 years with the mean age of 49.4 years. Preliminary slicing was done within 2 hours of surgery to allow adequate penetration of the fixative. The exision margins were marked with India ink. A detailed study on the morphological features was done with the specimen and the findings were recorded in a proforma. Emphasis was given to the size of tumor. The distance from the nearest excision margin was recorded. As many lymph nodes as possible was dissected from all the specimens.

Adequate tissues samples were taken from the tumor periphery, and resection margin closest to the tumor. Nodes less than 5mm in diameter were embedded intact, those greater than 5 mm were cut into slices not exceeding 5 mm. The tissues were embedded in paraffin blocks and 3-5 μm thick sections were cut in a rotary microtome and the slides were stained with conventional Haematoxylin and Eosin stain for the evaluation of histologic factors. Additional slides were subjected to special staining techniques with silver to assess the vascular invasion and to qualify the Argyrophilic Nucleolar Organizer Regions

(AgNORs) In randomly selected 20 cases estrogen receptor and progesterone receptor study was also undertaken, by immunohistochemical evaluation of paraffin embedded tissues using monoclonal antibody.

Staging was done based on American Joint Committee on Cancer System. The salient features of prognostic importance that were assessed include.

1) Architectural features

i) Tumor size

ii) Resection margin involvement

iii) Histological type

2) Invasive features

i) Vascular Invasion

ii) Lymph node deposits.

3) Proliferative features

i) Histological grade

ii) AgNOR

iii) Nottingham prognostic index.

The data obtained were recorded and tabulated in a proforma and were analyzed statistically.

RESULTS

Table 1 : Age

Age Group (in years)	Cases	
	No.	**%**
Less than 20	-	-
20-29	3	3.7
30-39	9	11.3
40-49	20	25.0
50-59	31	38.8
60 and Above	17	21.2
Total	80	100
Mean	**49.4 years**	

Table 1 Shows the incidence of breast carcinoma in different age groups in our study. The youngest was 24 years and the oldest was 76 years. The mean age was 49.4 years. Maximum number of cases (38.8%) were seen in the age group of 50 – 59 years; 85% cases were above 40 yrs.

Table 2 : Stage

Stage	Cases	
	No.	**%**
I	5	6.3
II	27	33.7
III	44	55.0
IV	4	5.0

Table 2 Shows the percentage of cases in each stage in our study. Maximum number of 44 cases (55%) were of stage III, followed by stage II with 27 (33.7%) cases.

Table 3 : Histological type

Histological type	Cases	
	No.	%
IDC	66	82.5
Lobular	6	7.5
IDC with comedo pattern	3	3.7
Others		
Lymphoma	1	1.3
Medullary	1	1.3
Papillary	1	1.3
Neuro endocrine	1	1.3
Mucinous	1	1.3
Others Total	5	6.3

IDC (NST) = Invasive Ductal Carcinoma (No Special Type)

Table 3 Shows the number of cases in each histological type of breast carcinoma. Maximum number of 66 cases (82.5%) were of IDC (NST). 6 cases (7.5%) were of lobular type, 3 cases (3.7%) were of IDC with comedo pattern. There was 1 case (1.3%) each of the other special sub types – Lymphoma, Medullary, Papillary, Mucinous and Neuroendocine carcinoma.

Table 4: Histological Grade

Histological Grade (grading done for 69 cases)	Cases	
	No.	%
I	4	5.8
II	38	55.1
III	27	39.1

Table 4 Shows the percentage of cases in each grade of invasive ductal carcinoma. Maximum number of 38 (55.1%) cases were of grade II, 27 cases (39.1%) were of grade III and 4 cases (5.8%) were of grade I tumor.

Table 5: ER, PR status

ER,PR	Cases	
	No.	%
ER+ PR+	5	25
ER+ PR-	2	10
ER- PR+	7	35
ER- PR-	6	30

ER = Estrogen Receptor ; PR = Progesterone Receptor

Table 5 Shows the ER, PR status in 20 breast carcinoma cases. 25% of cases were positive for both, while 30% of cases were negative for both. 35% of cases were positive for PR only and 10% of cases were positive for ER only.

Table 6: Lymph Node Stage

Lymph Node Stage	Cases	
	No.	%
Stage I	28	35
Stage II	33	41.4
Stage III	19	23.6

Table 6 Shows the percentage of cases in each lymph node stage. 35% cases were of lymph node stage I, 41.4% of cases of stage II and 23.6% of cases of stage III lymph node status.

Table 7: Nottingham Prognostic Index

NPI	Cases	
	No.	%
Good (≤ 3.4)	20	29
Moderate (3.5 – 5.4)	24	34.8
Poor (> 5.4)	25	36.2

NPI = Nottingham Prognostic Index

Table 7 Shows the percentage of cases belonging to each group of NPI score. 29% of cases were of good prognostic group, 34.8% were of moderate and 36.2% were of poor prognostic group.

Table 8 : Age and Stage

Stage	Age in years	
	Mean	S.D.
I	56.8	4.4
II	51.3	11.3
III	47.9	9.4
IV	40.8	11.7
'p'	**0.0319 (Significant)**	

Table 8 Shows the correlation between age of the patient and stage of the tumor.

Table 9 : Staging and Histological grade

Stage	Histological Grade (69 cases)					
	I		II		III	
	No.	%	No.	%	No.	%
I	3	100	-	-	-	-
II	-	-	21	100	-	-
III	1	2.4	17	40.5	24	57.1
IV	-	-	-	-	3	100

Table 9 Shows the correlation between TNM staging and modified Bloom and Richardson (Nottingham) grading. All of the stage I cases were of grade I and all of stage II disease were of grade II. Among stage III cases, 2.4% were of grade I, 40.5% were of grade II and 57.1% were of grade III. All the stage IV cases were of grade III.

Table 10 : Size and Grading

Grade	Size in cms	
	Mean	**S.D.**
I	3.08	2.62
II	5.55	2.13
III	8.2	2.62
'p'	**0.0001 (Significant)**	

Table 10 Shows average size of tumor in each grade of breast carcinoma. The mean size of tumor in grade I cases was 3.08 cm, grade II cases was 5.55 cm, grade III was 8.2 cm. This was found to be statistically significant.

Table 11 : Size and Lymph Node Status

Lymph Node Stage	Size in cms	
	Mean	**S.D.**
I	5.65	3.0
II	5.5	1.96
III	8.13	2.69
'p'	**0.0018 (Significant)**	

Table 11 Shows the correlation between size of the tumor and lymph node stage. The mean size of the tumor was 5.65 cm in lymph node stage I, 5.5 cm in stage II and 8.13 cm in stage III cases. This was found to be statistically significant.

Table 12. Resection Margin with Clinical Stage and Histological Grade

RM		Stage				Grade	
		I	II	III	IV	I	II
Involved	No.	-	3	14	3	1	5
	%	-	11.1	31.8	75	-	13.2
Free	No.	5	24	30	1	4	33
	%	100	88.9	68.2	25	100	86.8

RM – Resection Margin

Table 12 Shows the percentage of cases in each stage and in each grade with resected margin involvement. The resected margin was involved in 75% of stage IV case, 31.8% of stage III and 11.1% of stage II cases. The resected margin was involved in 13.2% of grade II and 48.1% of grade III cases.

Table 13 : Histological grade and Vascular Invasion

Histological Grade	Vascular Invasion			
	Present		Absent	
	No.	%	No.	%
I	2	50	2	50
II	19	50	19	50
III	19	70.4	8	29.6

Table 13 Shows the percentage of each grade of breast carcinoma with vascular invasion. Vascular invasion was present in 70.4% of grade III cases.

Table 14 : Vascular Invasion and Lymph Node Stage

Vascular Invasion	Lymph Node Stage					
	I		II		III	
	No.	%	No.	%	No.	%
Present	6	21.4	23	69.7	18	94.7
Absent	22	78.6	10	30.3	1	5.3
Total	28	100	33	100	19	100

Table 14 Shows the percentage of different lymph node stage cases with vascular invasion. Vascular invasion was present in 21.4% of lymph node stage I, 69.7% of stage II and 94.7% of stage III cases.

Table 15 : Histological Grade and Lymph Node Stage

Lymph Node Stage	Histological Grade					
	I		II		III	
	No.	%	No.	%	No.	%
I	2	50	17	44.7	5	18.5
II	2	50	19	50	6	22.2
III	-	-	2	5.3	16	59.3
Total	4	100	38	100	27	100

Table 15 Shows the correlation between lymph node stage and histological grade. 50% of grade I cases were of lymph node stage I and 50% of stage II. 50% of grade II cases were of stage II, 44.7% were of stage I and 5.3% were of stage III. Among grade III cases, 18.5% were stage I, 22.2% were stage II and 59.3% were stage III.

Table 16 : Stage and NPI

Stage	NPI	
	Mean	**S.D.**
I	1.97	1.24
II	3.57	0.96
III	5.62	1.67
IV	7.85	0.44
'p'	**0.0001 (Significant)**	

NPI = Nottingham Prognostic Index

Table 16 Shows the correlation between TNM staging and NPI of Invasive ductal carcinoma of Breast. The mean NPI was 7.85 for stage IV case, 5.62 for stage III, 3.57 for stage II and 1.97 for stage I cases. This was found to be statistically significant.

Table 17 : Stage and AgNOR

Stage	AgNOR	
	Mean	**S.D.**
I	2.52	0.11
II	2.84	0.19
III	3.2	0.26
IV	3.28	0.59
'p'	**0.0001 (Significant)**	

AgNOR = Argyrophilic Nucleolar Organizer Regions

Table 17 Shows the correlation between TNM staging and the mean AgNOR value. The mean AgNOR value was 2.52 in patients with stage I tumor, 2.84 in stage II, 3.2 in stage III and 3.28 in stage IV. This was found to be statistically significant.

Table 18 : Histological type and AgNOR

Histological type	AgNOR	
	Mean	**S.D.**
IDC [NST]	3.07	0.32
Lobular	2.7	0.2
IDC with comedo pattern	3.47	0.21
Others	2.78	0.28
'p'	**0.0023 (Significant)**	

AgNOR = Argyrophilic Nucleolar Organizer Regions

IDC [NST] – Invasive Ductal carcinoma (No Special Type)

Table 18 Shows the correlation between AgNOR and histological types of breast carcinoma cases. The mean AgNOR value was 3.47 in IDC with comedo pattern, 3.07 in IDC [NST], 2.7 in patients with Lobular carcinoma and 2.78 in patients with other special types. This was found to be statistically significant.

Table 19 : Histological grade and AgNOR

Histological Grade	AgNOR	
	Mean	**S.D.**
I	2.53	0.13
II	2.93	0.2
III	3.4	0.16
'p'	**0.0001** **(Significant)**	

AgNOR = Argyrophilic Nucleolar Organizer Regions

Table 19 Shows the correlation between the grade and AgNOR value of breast carcinoma cases. The mean AgNOR value for grade I cases was 2.53, grade II cases was 2.93 and for grade III was 3.4. This was found to be statistically significant.

Table 20 : AgNOR and NPI

NPI	AgNOR	
	Mean	**S.D.**
Good	2.85	0.27
Moderate	3.03	0.22
Poor	3.35	0.26
'p'	**0.0001** **(Significant)**	

NPI = Nottingham Prognostic Index
AgNOR = Argyrophilic Nucleolar Organizer Regions

Table 20 Shows the correlation between AgNOR and NPI. The patients were divided into 3 groups-Good, Moderate and poor prognostic groups based on the NPI score. The average AgNOR value in patients belonging to good prognostic group was 2.85, Moderate group was 3.03 and poor group was 3.35. This was found to be statistically significant.

DISCUSSION

Age :

The mean age of the patients included in our study was 49.4 years (Table 1). This is lesser than the observation made by Rhodes DJ, (2002), who found that more than 75% cases presented with the age of 50 and above and the mean age was 64 years.

Vinod.R, et al., (2005) had a similar observation of a mean age of 47 years, which correlates well with our study. These results indicate that carcinoma of breast in Indian subcontinent have a lower mean age compared with the western figures.

Age and stage :

In our study, we correlated the age of the patient to the clinical stage at presentation and we found that the mean age was high in patients with stage I disease when compared with the mean age of women with stage IV disease. It was also found to be statistically significant (p value = 0.0319) (Table :8).

This observation was similar to that of Foo CS, et al., (2005), who found that the younger patients have tumors with higher clinical stage and poorer prognostic profile. Jakic – Rezumovic J, et al., (2005) showed that there is statistically significant correlation between patients age and stage of disease with patients survival.

But younger women do not have a poorer overall survival due to the aggressive adjuvant therapy to which they are subjected.

Histological Types:

The correct classification of many of the histological types depends on the adequacy of tissue sampling.

A carcinoma is generally classified as invasive ductal carcinoma-No Special Type (NST) if the histological features of the special type constituted less than 10% and the tumor is termed as mixed if the special feature form between 10% and 90%. If the special histological features form more than 90% of the tumor area studied then the tumor is claimed as one of the special subtype. The identification of special histological subtype is of value due to the differences in tumor behaviour pattern of the various special types of breast carcinoma.

In our study, invasive ductal carcinoma (NST) constituted the highest number of cases (82.5%) of the total cases studied (Table :3).

This correlates well with the study of Dixon JM, et al., (1985. loc.cit.), who had reported an incidence of 79% in a study on the long term survival of breast cancer. In another larger series Ellis, et al., (1992. loc.cit.) assigned 47% of the tumor to this category.

Invasive lobular carcinoma contributed 7.5% of the total cases studied. This correlates with the observation of Dixon JM, et al., (1985. loc.cit.) who observed 10% of cases in his study.

Necrosis is not a prominent feature in most ductal carcinoma (NST). Rapidly growing densely cellular lesions may undergo intensive central necrosis producing a comedo pattern of necrosis. 3 cases (3.7%) of our study showed this specific histological pattern.

We had a single case of medullary carcinoma (1.3%) of the total cases analysed. This goes very well with the observation of Dixon JM, et al., (1985. loc.cit.) who found 2% of breast carcinoma had medullary pattern. The reported incidence of medullary carcinoma in various study was 5% (Mc Divitt RW, et al., 1968), 2.7% (Ellis IO, et al., 1992. loc.cit.), 1% (Sloane, et al., 1999). In our study the tumor fulfilled all the criteria for designation of typical medullary carcinoma laid down by Ridolfi, et al., (1977. loc.cit.).

Mucinous carcinoma constituted 1.3% of the total breast carcinoma studied. Sloane JP, et al., (1999. loc.cit.) found an incidence less than 5%, Dixon JM, et al., (1985. loc.cit.) observed an incidence of 2%. Ellis IO, et al., (1992. loc.cit.) found that the mucionus carcinoma has a better prognosis than infiltrating ductal and lobular carcinoma. He found an over all 10 year survival of 80%, a similar

observations was made by Toikanen S and Kujari H (1989).

Invasive papillary carcinoma constituted (1.3%) of the cases under our study. Most of the studies on the tumor have found an incidence of less than 1% (Sloane JP, et al., 1999 loc.cit).

We had one case of neuroendocrine carcinoma in a patient of 65 years. The mass was subareolar in location and presented with bloody nipple discharge. The tumor was composed of sheets of uniform small cells arranged in nests and cords with palisading.

Immunohistochemistry revealed positivity for synaptophysin. Papotti M, et al., (1989) concluded that most neuroendocrine carcinoma of breast exhibit positivity for synaptophysin. It is considered to be a more reliable histochemical marker. Scopsi L, et al., (1992) found positivity with chromogranin A or B in 86% of the cases and neuron specific enolase in 100% of cases, Andreola S, et al., (1988) have observed variable positivity for gastrin, insulin and bombesin.

Malignant lymphoma of the breast may be primary or more usually secondary. We had a single case of primary malignant lymphoma involving the breast. Mambo NC, et al., (1977) found 0.12% of cases and Bobrow LG, et al., (1993) 0.3% of cases. Even among the extra-nodal lymphomas, breast is an uncommon site. It mostly occurs in older women with a variable clinical course.

(Hugh JC, et al., (1990)). Immunohistochemical markers done on this case showed positivity for T cell marker.

Carbone A, et al., (1982) found a predominance of B cell pheno type in most cases. Cohen P and Brooks JJ (1991), Mattia AR, et al., (1993), had a similar observation.

The prognosis of primary lymphoma of breast has varied in different studies, but actuarial survival appears to be about 50% over 5 years. [De Cosse JJ, et al., (1962) ; mambo NC, et al., (1977) loc.cit]. Tumor size and axillary node involvement do not have the prognostic significance as in invasive ductal carcinoma (NST) tumors.

Histological type and AgNOR :

The correlation between histological type and AgNOR was done and we found that the mean AgNOR value in invasive ductal carcinoma with comedo pattern was highest (3.47), followed by Invasive ductal carcinoma (No special type) with a mean AgNOR of 3.07. Lobular carcinoma has the least mean AgNOR of 2.7. Carcinoma of other special types including medullary, mucinous, papillary and neuroendocrine carcinoma and lymphoma together had a mean AgNOR of 2.78 which is less than that of Invasive ductal carcinoma. This correlation has a significant p value of 0.0023 (Table : 18).

Our study is in concordance with the study by Aaltomaa S, et al., (1993), who correlated AgNOR with histological type and found it to be significant with a p value of 0.003.

Browser ST, et al., (1995) loc.cit suggested that in invasive tumors, comedo carcinoma are associated with poor prognostic factors. This correlates well with our observation. All the 3 cases of invasive ductal carcinoma (comedo) had a higher AgNOR value and higher mean NPI score.

Studies by Perez–Mesa CM, (1979), Ermilova VD, et al., (1990) loc.cit, Ellis IO, et al., (1992) loc.cit also confirm that histological typing of breast carcinoma can provide useful prognostic information.

Clinical stage and Histological grade:

Cases of invasive ductal carcinoma were alone included in this correlative exercise and the tumors were graded according to Nottingham modification of Bloom and Richardson system. We had 69 cases in this category (Table 9). Three cases of IDC presented as stage I lesion. The histological grade of these tumors revealed that all the 3 cases (100%) were of grade I malignancy.

There was an excellent correlation between the clinical staging and histological grading. All the 21 cases presenting as stage II disease, fell into the grade II category.

We had 42 cases presenting as stage III disease. Histological grading done on these cases reveal that majority of the cases (57.1%) were of grade III malignancy followed by 40.5% of cases of grade II malignancy and there was a single case present as grade I lesion.

All the 3 cases presenting as stage IV disease were found to be of higher histological grade of malignancy. This observation is similar to that of Rosen PP, et al., (1989) who observed significant increase in the histological grade of malignancy with advancing clinical stage. The other unfavourable factors could be increase in tumor size, perimenopausal status, number of involved axillary nodes, and presence of lymphatic or vascular emboli. So an analysis of the clinical stage and histological grade were complementary in assessing the prognosis of the case of carcinoma breast.

Size and grade:

In analysing the cases of breast carcinoma, we attempted a correlation between the histological grade of malignancy and the mean tumor diameter.

We found in grade I lesion the mean tumor size was 3.08 cm, for grade II lesions it was 5.55 cm and grade III lesions had a mean tumor size of 8.2 cm. These results were statistically significant with a p value of 0.0001 (Table : 10).

This correlates well with the observation of Sundquist M, et al., (1999) who found an accurate estimation of the tumor size together with the grade and lymph nodal status constituted statistically significant predictors of survival. So the parameter have been rightly incorporated in the calculation of Nottingham prognostic Index (Galea MH, et al., 1992. loc.cit).

Size and nodal status:

Axillary nodal involvement remain an essential prognostic factor for breast cancer patients, as it implies the necessity of systemic adjuvant treatment and loco regional radiation.

As tumor size and the lymphnodal status are independent prognostic variable in carcinoma breast we made an attempt to correlate the mean tumor size with that of the lymph node status.

The mean tumor size was 5.65 cm in patients with no detectable lymph nodes (stage I), the size was 5.5 cm in stage II cases and 8.13 cm in stage III group. The results were statistically significant with a p value of 0.0018 (Table : 11). This is in coherence with the observation made by Rudan I, et al., (1994), who analysed the various prognostic factors in patients with node negative breast cancer. They concluded that the tumor size and histological grade were significant prognostic parameter.

Cutuli. B, et al., (2001) found that the tumor size, and histological type were significant predictors of prognosis, which correlate well with our observation. Siddiqui T, et al., (2002) evaluated a series of helpful predictors of axillary node involvement. They found that the tumor size was one of the very significant predictor. The other predictors include age of menarche, duration of symptoms, and skin and nipple involvement. This is in support of our observation of a proportional increase in the tumor size with increase in the number of axillary nodes involved. Shen ZZ, (1991) studied a series of 2189 cases of radical surgery and showed a linear relation between tumor size and percentage of case with positive lymph node involvement. Similar results were observed by Barranger E, et al., (2005).

Rack B, et al., (2003) had a similar observation in case of recurrent breast cancer and he found a significant correlation between the tumor size and nodal status.

Vascular invasion with Grade and Lymph Node Stage:

High grade malignant tumors have a shorter tumor doubling time, are less cohesive and often with irregular borders and tend to invade by small aggregates of tumor cells. The presence of vascular invasion provides considerable information on the aggressiveness of the neoplasm. We tried to incorporate this hypothesis in our study and we correlated the incidence of vascular invasion with

that of the histological grade and lymph node stage of tumors. Vascular invasion was observed in 47 case (58.8%) and was absent in 33 cases (41.2%). 69 cases of IDC were included in this study of incidence of vascular invasion in each grade of breast carcinoma. The percentage of vascular invasion was higher in grade III malignancy (Table: 13). Vascular invasion was seen in 21.4% of stage I lymph node status and increased with the increase in number of nodes involved (Table: 14).

This observation correlates well with that of Pinder SE, et al., (1994) loc.cit who found that the vascular invasion was strongly associated with lymph node stage, tumor size, histological grade and histological type of the tumor.

Hartveit F, et al., (1984), demonstrated that the presence or absence of tumor cells in the efferent nodal vessels can be used as a measure of nodal stage.

In the analysis done by Pinder SE, et al., (1994) loc.cit in a series of 1704 invasive breast carcinoma cases, vascular invasion was strongly associated with lymph node stage which was statistically significant with a p value of < 0.001. Blood vessel invasion and axillary lymph node involvement were examined by Weigand RA, et al., (1982. loc.cit.). They suggested that blood vessel invasion in combination with node status, is a prognostic indicator with high discriminatory power.

The study by Centintas SK, et al., (2006) shows that the presence of lymphatic vessel invasion increased the risk of axillary node involvement with a p value of 0.0003.

Resection Margin with clinical stage and Histological grade :

Assessment of surgical resected margin is commonly used as a guide to the relative aggressiveness of therapy and to predict the rate of relapse.

In our study, resected margin was free in all the case of stage I breast cancer and majority of 24 cases (88.9%) had free margins in stage II. This was reduced to 30 cases (68.2%) in case of stage III disease and 75% of stage IV cases had involvement of the resection margin (Table 12).

Wager DE, et al., (1997. loc.cit.) had a similar observation while evaluating the status of the resection margins as a predictor of residual tumor burden.

A similar correlation was done between the histological grade of the tumor and the status of the resection margin. The invasive ductal carcinoma were only included in this correlative exercise and we found resected margin was free in all the 4 case of grade I malignancy. 33 cases (64.7%) of grade II malignancy had free margin and only 14 cases (27.5%) of grade III malignancy had free margin.

This clearly indicates that, as the histological grade of malignancy increases, the involvement of the resection margin also increases and contribute to one of the prognostic predictors in cases of carcinoma breast.

Lymph Node Status and Grade:

Axillary node involvement is an important prognostic variable in the management of patients with primary breast cancer. It is a time - dependent variable but the studies by Jatoi I, et al., (1999. loc.cit.) and Rack B, et al., (2003. loc.cit.) showed that it is also a marker of an aggressive phenotype.

We correlated lymph node stage with the histological grade of the tumor. Among grade I cases 50% were of lymph node stage I and 50% were of stage II. Among grade II cases, maximum number of cases (50%) were of stage II nodal status and 5.3% of cases were of stage III nodal status. Most of grade III cases (59.3%) fell into stage III lymph node status (Table 15).

Rudan I, et al., (1994. loc.cit.) concluded that among stage I patients, grade and tumor size can serve as helpful predictors of 5-year overall survival. Similar observations were made by Nottage MK, et al., (2006).

Nottingham Prognostic Index (NPI) :

The NPI is an integrated prognostic index used to predict patient survival for women with invasive breast cancer. We have calculated the NPI in all our cases (Table: 7).

In our study, 20 cases (29%) had a good NPI score, 24 cases (34.8%) had an average score and 25 cases (36.2%) had poor NPI scores.

These scores were then correlated with the clinical stage of the disease. We have found a NPI score of 1.97 in stage I, 3.57 in stage II, 5.62 in stage III and 7.85 in stage IV (Table :16). This very well correlates with the studies of Kollias J, et al., (1999), Haybittle JL, et al., (1982), Galea MH, et al., (1992), and Okugawa H, et al., (2005) who suggested an observation of higher the score worser is the prognosis.

We also correlated the NPI with the AgNOR values. All the cases with a good NPI had a low AgNOR score and patients with poor NPI had a higher AgNOR score (Table: 20).

This correlates well with the observation of Subramanian S, et al., (1996), who found that the AgNOR count was significantly related to the cell size, histological grade and the presence of nodal metastasis. Calculation of the NPI and AgNOR is of immense value in predicting the prognosis and disease free survival

status.

The correlation between vascular invasion and NPI score was also significant with a p value of 0.0001. The mean NPI score in tumors with vascular invasion was 5.85 and in tumors without vascular invasion was 3.74.

Stage and AgNOR :

The relation between TNM staging and argyrophilic nucleolar organizer regions (AgNOR) within human breast cancer cells was analyzed. The mean AgNOR count for stage IV was highest (3.28) and that for stage I was low (2.52). Stage II and stage III tumor had intermediate value of 2.84 and 3.2 respectively. This correlation was significant with a p value of 0.0001(Table 17).

Aubele M, et al., (1994) investigated the correlation of AgNOR features with other prognostic variable and found that TNM staging showed significant correlation with AgNOR count.

Kumar A, et al., (1997) evaluated AgNORs in 46 patients with primary breast carcinoma and correlated these with clinical prognostic parameters. Their results shows a statistically significant increase in correlation with increase in the size of the tumor, stage of the cancer and number of metastatic lymph nodes.

These results indicate that breast tumors with a higher AgNOR count, even at the initial stage, have a poor prognosis and require aggressive treatment for better control of the disease.

Grade and AgNOR:

The relevance of silver stained NORs for prognosis in breast cancer was investigated by correlating it with modified Bloom– Richardson (Nottingham) grading. The mean AgNOR count in grade III tumors was 3.4 which fell to 2.93 in grade II and 2.53 in grade I tumors with a significant p value of 0.0001 (Table 19).

Aubele M, et al., (1994.loc.cit.) found significant differences in AgNOR score with Bloom–Richardson gradings I, II and III. Ofner D, et al., (1996) suggested that AgNOR count was statistically significantly correlated with histological grade and hormone receptor status of tumors. Increase AgNOR count was statistically significantly associated with early tumor relapse and cancer related death.

Aaltomaa S, et al., (1993. loc.cit.) suggested that the AgNOR count was related significantly to histological grade [$p < 0.001$) histological type, and hormone receptor content. But Gupta GR, et al., (1997) found no correlation between AgNOR and histological grade while it correlated with tumor size and axillary lymph node metastasis.

In our study AgNOR count correlated well with pattern of vascular invasion. The mean AgNOR count in tumors with vascular invasion was 3.11 where as the same in tumors without vascular invasion was 2.95. This was found to be statistically significant with a p value of 0.244.

Estrogen Receptor and Progesterone Receptor Status (ER/PR):

The hormone receptor status had no statistically significant correlation with the other prognostic parameters like grade, lymph nodal status and vascular invasion. Roberts AN, et al., (1981) had a similar observation.

Collett K, et al., (1998), Aamdal S, et al., (1984) loc.cit have suggested that the ER/PR status can be considered as an independent factor, more of therapeutic importance rather than predicting the prognosis.

However inclusion of the evaluation of ER/PR status along with other proliferative parameters is more beneficial to the patients to assess the nature of adjuvant chemotherapy.

CONCLUSION

The role of histopathology in the provision of prognostic information has been well established in a number of malignant tumors.

Mammographic screening detects breast cancers which are different in biological behaviour from those which present symptomatically. Further, there has been an increase in the number of treatment options, both surgical and medical. The attitude of patients is changing and more women are exercising their right to participate in the management and therapeutic decisions.

These developments highlight the importance of evaluation of prognostic factors in the management of patients with breast cancer. The most important reason for the use of prognostic factors are to identify patients.

- Whose prognosis is so good that adjuvant therapy after local surgery would not be cost-beneficial.
- Whose prognosis is so poor that a more aggressive adjuvant therapy would be warranted.
- Who are likely to respond to particular types of therapy.

In our study we evaluated the prognostic significance of histological typing, staging, grading, tumor size, vascular invasion, resection margin involvement, AgNOR and NPI.

There was statistically significant association between these individual prognostic parameters.

So we conclude that a proper evaluation of the morbid anatomy, exact tumor size, clinical stage, histological grade, histological subtype lymph node status and margins are mandatory for prognostication.

Additional parameters like the NPI and AgNOR can be done along with the above parameters to give a comprehensive report regarding the patients prognosis.

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PROFORMA – A

MACROSCOPIC STUDY

Name & Age of the Patient :

I.P. No. :

Path Ref. No :

Type of specimen :

Whole size of the specimen : cm

Maximum diameter of tumour : cm

Disease Extent :

Excision Margin :

Overlying skin :

Nipple & Areola :

Number of lymph nodes :

Size – Largest :

Smallest :

Stage of the tumour :

PROFORMA - B

MICROSCOPIC FEATURES

1. Histological subtype :

2. Histological grade :

3. Tumour size :

4. Resection Margin Involvement :

5. Vascular Invasion :

6. Lymph node status

    Total No. of nodes :

    Positive :

    Negative :

7. Quantification of AgNORs :

8. Calculation of Nottingham

    Prognostic Index :

9. ER/PR status :